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Quantifying the forces driving cell-cell adhesion in a fungal pathogen

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Abstract

Owing to its ability to form biofilms on implanted medical devices, the fungal pathogen *Candida albicans* causes frequent infections in humans. A hallmark of *C. albicans* biofilms is the presence of two types of cells, budding yeast cells and growing hyphae, which are bound together and embedded in extracellular matrix material. Although cell-cell adhesion is critical to biofilm formation, architecture and cohesion, we know little about the fundamental forces behind this interaction. Here, we use single-cell force spectroscopy (SCFS) to quantify the forces engaged in yeast-hyphae adhesion, focusing on the role of Als (Agglutinin-like sequence) proteins as prototypes of cell adhesion molecules. We show that adhesion between individual yeast and hyphal cells involves strong, short-range cohesive interactions (1.1 nN ± 0.2 nN; 86 ± 33 nm), and weak, long-range tether interactions (0.4 ± 0.2 nN; 234 ± 81 nm). Control experiments demonstrate that these interactions originate from cell surface proteins that are specific to *C. albicans*. Using mutant strains deficient for Als expression, we find that Als3 proteins, primarily expressed on the germ tube, play a key role in establishing strong cohesive adhesion. We suggest a model in which cohesive adhesion during biofilm formation originates from tight hydrophobic interactions between Als tandem repeat domains on adjacent cells. When subjected to force, the two interacting cell surfaces detach but the cell bodies remain tethered through macromolecular extensions. Our results represent the first direct, non-invasive measurement of adhesion forces between interacting fungal cells, and provide novel insights into the molecular origin of the cohesive strength of fungal biofilms.

Keywords

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INTRODUCTION

The formation of biofilms on tissues and implanted devices is responsible for a wide range of microbial infections.[1,2] Bacterial and fungal biofilms consist of microcolonies made of cell aggregates and enclosed in a matrix of extracellular polymeric material. Although cell-cell interactions play key roles in controlling biofilm cohesion and architecture, their molecular details are poorly understood.

A remarkable example of biofilm-forming microbe is *Candida albicans*, a common fungal pathogen found in hospital settings and in chronic disease patients, and which can lead to both morbidity and mortality.[3,4] An important feature of *C. albicans* biofilms is the presence of two morphological forms of the fungus: budding yeast cells and growing hyphae.[3] The development of a biofilm starts with the formation of an initial basal layer made of yeast-phase cells adhering to a substrate. Formation of microcolonies and germination of yeast cells then leads to the addition of an upper hyphal layer. The two fungal forms display major differences in their cell surface macromolecules, including the cell adhesion glycoproteins known as Als (<u>A</u>gglutinin-<u>l</u>ike <u>s</u>equence) proteins,[5,6] and are believed to play distinct roles in biofilm formation. To date, how fungal morphogenesis modulates cell-cell adhesion and cohesion in *C. albicans* biofilms is poorly understood. Clarification of this issue is critical to our understanding of the molecular bases of biofilm formation and may contribute to the development of new antifungal therapies.

Among the eight different Als proteins produced by *C. albicans*, Als1 and Als3 are considered as the most important for biofilm formation.[7,8] Als3 expression is induced in the initial stages of biofilm formation,[7] and is primarily localized in germ tubes and hyphae.[9–11] Deletion of the *ALS3* gene strongly affects *in vitro* biofilm formation, but not under *in vivo* conditions, probably due to higher expression of the homologous gene *ALS1* under these conditions.[9,10,12] All Als proteins share three distinct functional regions that are engaged in cell adhesion. The two N-terminal immunoglobulin (Ig)-like regions show broad substrate specificity and initiate cell adhesion. These are followed by a threonine-rich region (T) containing a 7-residue sequence that strengthen cell adhesion through amyloid bonds. The central region of the protein contains a variable number of tandem repeat (TR) domains that are 36 amino acids in length, and bind to each other and to various substrates through hydrophobic interactions. Previously, we used single-molecule atomic force microscopy (AFM) to demonstrate that these three regions mediate strong recognition binding events (Ig region), amyloid-mediated clustering and interactions (T region), and strong hydrophobic interactions associated with protein unfolding (TR region).[11,13–15] Yet, the extent to which these different Als-based interactions contribute to the adhesion of whole cells is unknown.

Traditional methods used in microbiology provide averaged information obtained on large populations of cells. By contrast, the emerging field of single-cell microbiology uses new tools to analyze individual cells in complex, heterogeneous populations, thereby enabling us to reveal a diversity of behaviors and rare events that would otherwise be hidden.[16,17] Among these technologies, atomic force microscopy (AFM) has been instrumental in unravelling the structure, properties and interactions of living cells at the single-cell and single-molecule levels.[18,19] In the cell adhesion context, AFM-based single-cell force spectroscopy (SCFS) has proven very useful for measuring the fundamental forces driving cell-substrate and cell-cell adhesion.[20–23] The general principle is to immobilize a single living cell on an AFM cantilever and to measure the forces between this cell probe and a substrate or another cell. Here we use SCFS for quantifying the forces engaged in yeast-hyphae adhesion in *C. albicans*. We demonstrate that the adhesion between individual yeast and hyphal cells is mediated primarily by Als3 adhesins expressed on germ tubes, and involves strong, short-range cohesive interactions and weak, long-range tether interactions.

The method presented here is a powerful tool in single-cell microbiology for quantifying the adhesion forces between different fungal morphotypes (yeast *vs* hyphae), thereby contributing to increase our understanding of the mechanisms of biofilm formation.

RESULTS AND DISCUSSION

Probing cell-cell adhesion

Cell probes were prepared by attaching single fungal cells on tip-less cantilevers using polydopamine.[24,25] An important issue in SCFS is the standard tilt (~10°) of the cantilever, which can cause non-uniform, tangential load on the cell, and, in turn, cell sliding and rolling.[26] To circumvent this problem, cells were attached on wedged cantilevers prepared using the protocol of Stewart *et al.* (Figure 1a).[26] Wedged-cantilevers were coated with a thin film of polydopamine.[24,25] Using an integrated AFM-inverted optical microscope, the polydopamine probes were then approached toward a single yeast cell deposited on a glass petri dish in buffer, kept in contact for 10 s, and then withdrawn (Figure 1b).

To probe the adhesion between single yeast and hyphal cells, a yeast cell probe was approached towards an hyphal cell immobilized on a hydrophobic substrate.[11] This immobilization method may select for a certain subpopulation of cells, but this was the only approach available to firmly attached hyphal cells without using chemicals that could alter the cell surface. As shown in Figure 1c,d, the set-up is better visualized by labelling the cells with green ConA-FITC (yeast cells) and blue Calcofluor White (hyphae). Note that to avoid interference with the labelling molecules, the cells were not labelled for SCFS measurements. The use of an integrated AFM-inverted optical microscope enabled us to accurately position the yeast probe over the yeast (Figure 1c) or the germ tube (Figure 1d) regions of the hyphae cell. The viability of the cells was checked using a LIVE/DEAD yeast viability staining protocol.[27] Fluorescence images showed that both yeast and germinating cells were alive before and after the SCFS experiments (Supporting Information Figure 1, red color), thus confirming that cell probe preparation and SCFS measurements were minimally invasive.

Representative force-distance curves recorded between individual yeast and hyphal cells are shown in Figure 1e,f. While the curves obtained on the yeast region of the hyphae showed no adhesion (Figure 1e), those recorded on the germ tube featured large adhesion forces with multiple peaks and extended rupture lengths (Figure 1f). These force profiles indicate that the molecular bonds formed between the two cells break sequentially until they have completely separated from each other.[20,22]

Quantifying the adhesion forces between yeasts and hyphae

To gain insight into the molecular origin of the measured adhesion forces, force profiles obtained for multiple cells were examined in detail, and the relevant parameters compiled into histograms and scatter plots (Figure 2). As shown in Figure 2a,b, the curves recorded at a loading rate (LR) of 120.000 pN/s between yeasts and hyphae showed two characteristic features, *i.e.* a large adhesion force peak (F_{max} = 1.1 nN ± 0.2 nN; mean ± standard deviation; n = 500 curves from 5 independent cell combinations; 92 % of the curves;) at short distance (86 ± 33 nm), that was often followed by a second peak of weaker adhesion (F_{teth} = 0.4 ± 0.2 nN; 61 % of the curves) at longer distance (234 ± 81 nm). The histograms in Figure 2c,d demonstrate that the general features of the curves did not substantially change when recording consecutive force curves or when comparing different cells. To correlate the force and distance values for each F_{max} and F_{teth} peaks, both parameters were plotted in scatter plots (Figure 2e,f), and in bivariate diagrams (insets) in which the probability density is displayed as a color map (low and high densities are shown in blue and

red, respectively). From these plots, it can clearly be seen that the strong adhesion events (F_{max}) occur at short distances, while most weak adhesion events (F_{teth}) are of much longer range. These observations lead us to conclude that strong adhesion events represent the largest cell- cell binding strength at close contact, referred to as "cohesive interactions", while weaker events correspond to extended macromolecular bonds, referred to as "tether interactions", occurring after the two cell bodies have detached from each other.[21,22]

Control experiments were performed to determine whether cohesive interactions originate from cell surface proteins that are specific to *C. albicans*. Force curves recorded between non-pathogenic yeast *Saccharomyces cerevisiae* and *C. albicans* hyphae yielded a very low adhesion frequency (8 %, Figure 3a,b; see red color in the lower left corner of the probability density map), and the adhesion profiles showed only weak tether interactions ($F_{max} = F_{teth} = 0.4 \pm 0.1$ nN; 257 ± 86 nN; LR = 120.000 pN/s). In addition, treatment of *C. albicans* hyphae with urea (4 M) led to similar results (Figure 3c,d; $F_{max} = F_{teth} = 0.3 \pm 0.2$ nN; 222 ± 132 nN), thus indicating that disruption of hydrogen bonds in cell surface proteins abolish cell-cell adhesion. These observations lead us to conclude that the measured yeast-hyphae interactions require *C. albicans* surface proteins.

Als proteins on the germ tube largely contribute to cell-cell adhesion

Als glycoproteins play key roles in mediating adhesion and biofilm formation in *C. albicans*. Although some Als, like Als3, are known to be primarily expressed on germ tubes, it is unclear whether changes in Als expression during fungal morphogenesis modulate cell-cell adhesion. To address this issue, we measured the forces between WT yeast cells and germ tubes from the double mutant *als3Δ/als3Δ als1Δ/als1Δ* deficient for Als3 and Als1 expression (Supporting Information Figure 2a; Figure 4a,b; LR = 120.000 pN/s). Force signatures of the double mutant were very different from those of the WT. First, the adhesion probability (i.e. fraction of force curves showing adhesion events) dropped from 92 % to 66 % (red color in the lower left corner of the probability density map). Second, most adhesion curves showed only weak tether events ($F_{max} = F_{teth} = 0.6 \pm 0.5$ nN; $n = 300$ curves) with extended rupture distances (279 ± 117 nm), while strong/short cohesive events were essentially missing. These observations demonstrate that Als3 and (or) Als1 plays a crucial role in establishing strong cohesive adhesion between yeast and hyphal cells.

To clarify the respective roles of Als1 and Als3 in yeast-hyphae adhesion, we analyzed germ tubes from the *als1Δ/als1Δ* (Figure 4c,d) and *als3Δ/als3Δ* (Figure 4e,f) single mutants (Supporting Information Figure 2b,c). We found that knockout of Als1 alone (*als1Δ/als1Δ* mutant) leads to almost full recovery of the cohesive interactions. Most curves (93 %) showed a strong adhesion peak ($F_{max} = 1.5 \pm 0.4$ nN; n=300 from 3 independent yeast/ hyphae) at short distance (94 ± 13 nm), generally followed (65 %) by a second peak of weaker adhesion ($F_{teth} = 0.7 \pm 0.5$ nN) at longer distance (190 ± 75 nm), a behavior very similar to that of the WT. Notably, expression of Als1 alone (*als3Δ/als3Δ* mutant) also led to the reappearance of some cohesive adhesion peaks ($F_{max} = 0.7 \pm 0.1$ nN; 93 ± 23 nm) but these were less frequently observed (68 %) and of lower magnitude. These results show that the localized accumulation of Als3 proteins - and to a lesser extent Als1 proteins - on hyphae is the main driving force of the yeast-hyphae adhesion.

Als3 proteins provide remarkable cohesive strength to cell-cell adhesion bonds

To better understand the molecular nature of Als3-mediated cell-cell adhesion, we calculated the work of adhesion (or detachment) from the area under the retraction curves.[20,28] Figure 5 shows the variation of the cumulative work of adhesion as a function of the distance, calculated for the WT and for the single mutants (averaged from 20 force curves recorded on 3 different cells). For the WT, it can clearly be seen that strongly

adhesive interactions contribute to most of the work of adhesion (up to ~180 10^{-18} J) at short distances (0–200 nm), while weak tether interactions only have a marginal contribution (~25 10^{-18} J) at longer distances. While the *als1Δ/als1Δ* mutant shows the same behaviour as the WT, the *als3Δ/als3Δ* mutant displays a dramatic decrease of work of adhesion (~50 10^{-18} J).

Can we relate the work of adhesion to a surface density of interacting Als molecules? The work of adhesion of a single Als protein can be estimated from earlier single-molecule measurements[13,14] to be ~20 aJ (estimated from single-molecule experiments performed at similar temperature and with a loading rate of 100.000 pN/s), meaning that the ~200 aJ value corresponds to the detachment of ~10 molecules. Note that this calculation assumes parallel loading of all bonds and linearly-additive adhesion forces, which may not be strictly correct for such single-live cell experiments. Nevertheless, the obtained values may be converted into protein surface densities, considering the cell-cell contact area. As a rough approximation, the contact zone of two deformable spheres pressed on each other may be estimated by the following Hertz model equation: $A = \sqrt[3]{\dfrac{3FR}{4E^*}}$ in which A is the radius of the contact area, R the effective radius ($1/R = 1/R_1 + 1/R_2$), F the applied load, and E^* the effective young modulus ($1/E^* = ((1-v_1^2)/E_1) + ((1-v_2^2)/E_2)$ in which E_1, E_2 are the elastic moduli and v_1, v_2 the Poisson's ratios associated with each body). Considering a radius of 1.5 μm, a young modulus of 0.6 MPa (determined from indentation measurements) and a Poisson's ratio of 0.3 for the yeast cell and a radius of 0.6 μm, a young modulus of 7 MPa and a Poisson's ratio of 0.3 for the germ tube, we found an area radius of ~100 nm, thus yielding a contact area of ~0.03 μm². The number of molecules in interaction in this area gives a surface density of ~320 Als/μm², a value in the range of the average cell surface concentration expected for *Candida* adhesins. Altogether, these observations demonstrate that Als3 provides strongly cohesive adhesion energy between yeast and hyphae cells, which is likely to play an important role in biofilm formation.

We suggest that yeast-hyphae cohesive adhesion originates from tight hydrophobic interactions between Als TR domains on adjacent cells, while tether adhesion would involve the terminal Ig domains on fully stretched Als proteins. Supporting this view, we observed that addition of Ig-ligand peptide "KLRSMAYKIPTHRR" to WT hyphae had a moderate effect, i.e. slight decrease of adhesion frequency and of the maximum adhesion force, suggesting that Ig domains are important for proper extension of the TR regions (Supporting Information Figure 3a); by contrast, addition of the peptide to the double mutant *als3Δ/als3Δ als1Δ/als1Δ* completely abolished all adhesion signatures, including tether interactions, confirming that these events are mediated by the Ig region (Supporting Information Figure 3b).

CONCLUSIONS

We have shown that the non-destructive attachment of single yeast cells to AFM cantilevers using polydopamine is a valuable method for measuring cell-cell adhesion forces in fungal pathogens. The use of wedged cantilevers makes it possible to avoid tangential load on the cell, thus cell sliding and rolling problems. Using this single-cell technology, we have provided the first direct measurement of adhesive forces between interacting fungal cells. We demonstrated that yeast-hyphae adhesion involves both strong (short-range) cohesive interactions and weak (long-range) tether interactions that are essentially mediated by Als3 proteins expressed on the germ tube. Our experiments provide direct evidence that hyphal formation results in stronger cell-cell interactions, thus that hyphal transition is important for

strong biofilm formation. This finding explains why mutants altered in the yeast-to-hyphae transition are strongly affected in their ability to produce thick biofilms.[29]

Our results favor a model in which strong cohesive adhesion between yeast and hyphae originates from tight hydrophobic interactions between Als TR domains on adjacent cells, i.e. Als3 domains on hyphae and other domains like Als1 or Als5 domains on yeasts (Figure 5c). This mechanism is consistent with the notion that germ tubes are highly hydrophobic,[11, 30] and that cell surface hydrophobicity is important for *C. albicans* adhesion.[30–32] Upon cell-cell separation, force-induced unfolding of the TR domains may enhance hydrophobic interactions through increased exposure of hydrophobic residues, up to distances of ~200 nm corresponding to fully stretched TR domains. Further separation would lead to detachment of the interacting TR domains, while the cells would remain connected through weak tether interactions, a phenomenon which increases the lifetime of the adhesive bond (Figure 5d).[22] As tether bonds were rarely observed in control conditions (germinating yeast cell, germ tube treated with urea or probed with a *S. cerevisiae*), we believe they involve binding of the terminal Ig domains of fully stretched Als3 to their cognate ligands, i.e. peptide sequences containing the "τφ+" motif. In natural biofilms, we expect that Als3 Ig domains will bind to other Als proteins but also to Hwp1 and related cell surface proteins found on *C. albicans* hyphae. It has indeed been shown that, whereas *als1Δ/als1Δ als3Δ/als3Δ* or *hwp1Δ/hwp1Δ* mutants were not able to form biofilms, mixing of such mutant cells resulted in full biofilm formation, suggesting that interaction between the Als and Hwp1 proteins is important for cell-cell interactions and biofilm formation.[22]

In our cell-cell experiments, one cell (the yeast cell attached to the cantilever) had a sparse surface expression of adhesins, while the other (the germ tube) produced much more adhesins.[11, 33] Under these conditions, we predict that a substantial fraction of the probed interactions are between Als adhesins on the hyphae and non-Als proteins on the yeast cell (Figure 5). Indeed the decreased interactions between yeast cells and *als* mutant hyphae supports this interpretation. Although we do not know the exact identities of the interacting molecules on the yeast cells, hydrophobic cell wall mannoproteins are surely involved.[30] One candidate would be Eap1, a hydrophobic mannoprotein expressed on *C. albicans* yeast cells. The mature form is composed of about 600 amino acid residues in tandem repeats and it mediates binding to polystyrene.[34] Eap1 also displays multiple copies of the "τφ+" motif that binds to Als Ig-like regions, including a pair in tandem near the N-terminus.[35] Therefore, this protein would participate in the bulk hydrophobic interactions involved in cohesive adhesion (Figure 5c). The N-terminal position "τφ+" sequences would allow maximal extension before bond rupture (Figure 5d). We anticipate that the interactions between pairs of highly adhesive cells, e.g. two hyphal cells, would be even stronger than what we measured here. Such strength of adhesion explains the high resistance of *C. albicans* biofilms to disruption by shear.[36,37]

METHODS

Microorganisms and cultures

C. albicans SC5314 cells were cultivated on YPD medium (1% yeast extract, 2% Bacto-peptone, 2% D-glucose, supplemented with 2% agar) at 37°C.[38] Few colonies were inoculated in YPD liquid medium and agitated overnight (30°C, 200 rpm). Non-germinated cells were harvested by centrifugation, washed 3 times with sodium acetate buffer and resuspended in 10 mL buffer to a concentration of ~10^6 cells/mL. For hyphae formation, germination was induced by inoculating 250 μL of cell suspension in 8 mL of RPMI 1640 medium buffered with MOPS (Sigma) at pH 7, and agitated at 37°C, 200 rpm, for 90 min unless otherwise stated. We used mutant strains with deletion of both alleles of ALS genes (kindly provided by Aaron Mitchell, Carnegie Mellon University, Pittsburgh, PA).[8] These

mutants were derived from CAI-438 and included the single mutants *als1Δ/als1Δ* (Δals1)[30] and *als3Δ/als3Δ* (Δals3)[12] and the double mutant *als3Δ/als3Δ als1Δ/als1Δ* (Δals3Δals1).

Fluorescence microscopy

Following germination for 90 min in RPMI, cells were washed twice by centrifugation in buffer. Cells were resuspended in 20 μL/mL were incubated in a 25 μM solution of Calcofluor White M2R for 30 min at 37°C. Non-germinated yeast cells were washed twice by centrifugation in buffer. Cells were resuspended in 20 μL/mL in acetate buffer supplemented with 1mM Ca^{2+} and 1mM Mn^{2+} and incubated in a 30 μg/mL solution of fluorescein isothiocyanate (FITC)-conjugated Concanavalin A for 30 min at 37°C. Images were acquired with a Zeiss Axio Observer Z1 equipped with a Hamamatsu camera C10600.

Viability tests

The viability of yeast and hyphal cells was tested using a LIVE/DEAD yeast viability kit (Molecular Probes). Prior to attachment, a cell suspension (10^6 cell/mL in 2% glucose Hepes solution) was mixed with FUN1 cell stain (5 μM), mixed thoroughly and incubated for 30 min in the dark at 30°C. Labelled cells were then attached to polydopamine probes or solid substrates, and their viability checked by fluorescence microscopy.

Atomic force microscopy

AFM measurements were performed at room temperature (20°C) in sodium acetate buffer using a Bioscope Catalyst from Bruker corporation (Santa Barbara, CA). Germinating cells were immobilized through hydrophobic attachment on solid substrata. To this end, glass coverslips coated with a thin layer of gold were immersed overnight in a 1 mM solution of 1-dodecanethiol (Sigma), rinsed with ethanol and dry under N_2. After induction of germ tube formation in RPMI, the cells were harvested and rinsed three times in acetate buffer. Drops (200 μL) of the concentrated suspension were deposited on the hydrophobic substrates and let to stand for 3 h. After rinsing the unattached cells with buffer, the forces between yeast cell probes (see below) and immobilized hyphae were measured. Force curves were recorded with a maximum applied force upon approach of 1 nN, using a constant approach and retraction speed of 1000 nm.s^{-1} and a surface delay of 1 s. For blocking experiments, 0.2 mg/mL of "KLRSMAYKIPTHRR" peptides (Eurogentec) were injected in the solution 30 min before the experiment.

Cell probes

We used tipless, oxide sharpened microfabricated cantilevers with a nominal spring constant of ~0.12 N/m (Microlevers, Veeco Metrology Group). The spring constants of the cantilevers were measured prior to the experiments using the thermal noise method. For cell probe preparation, a tipless cantilever was immersed for 1 h in a 10 mM Tris buffer solution (pH 8.5) containing 4 mg/mL dopamine hydrochloride (99%, Sigma-Aldrich). The cantilever was then washed and dried under N_2, brought into contact with an isolated non-germinated cell for 10 s, and the obtained cell probe was then transferred without dewetting over a germinated cell for further force measurements.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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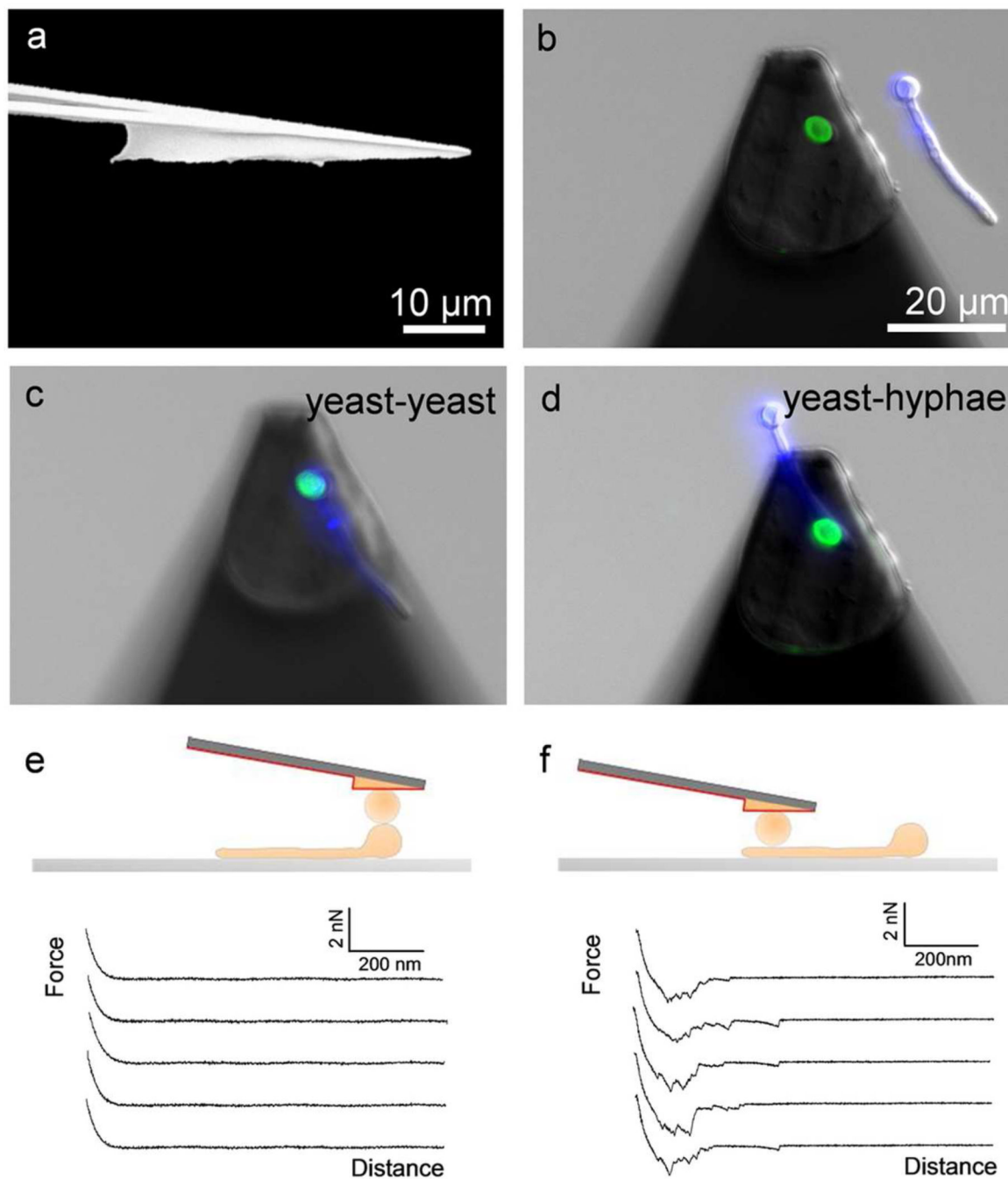
Figure 1.
Force spectroscopy of cell-cell adhesion using wedged cantilevers. (a) Scanning electron microscopy image of a wedged cantilever prepared using UV-curable glue. (b) Non-destructive method for studying yeast-hyphae adhesion: single yeast cells from *Candida albicans* (labeled with Con A-FITC, green) were attached on a polydopamine-coated wedged cantilever and approached towards a *C. albicans* hyphae (Calcofluor White, blue) immobilized on a hydrophobic substrate. (c-f) To measure yeast-hyphae adhesion forces, the yeast cell probe was positioned on top of the yeast region (c, e) or the germ tube region (d, f) of the hyphae, and multiple force-distance curves were recorded (e, f).
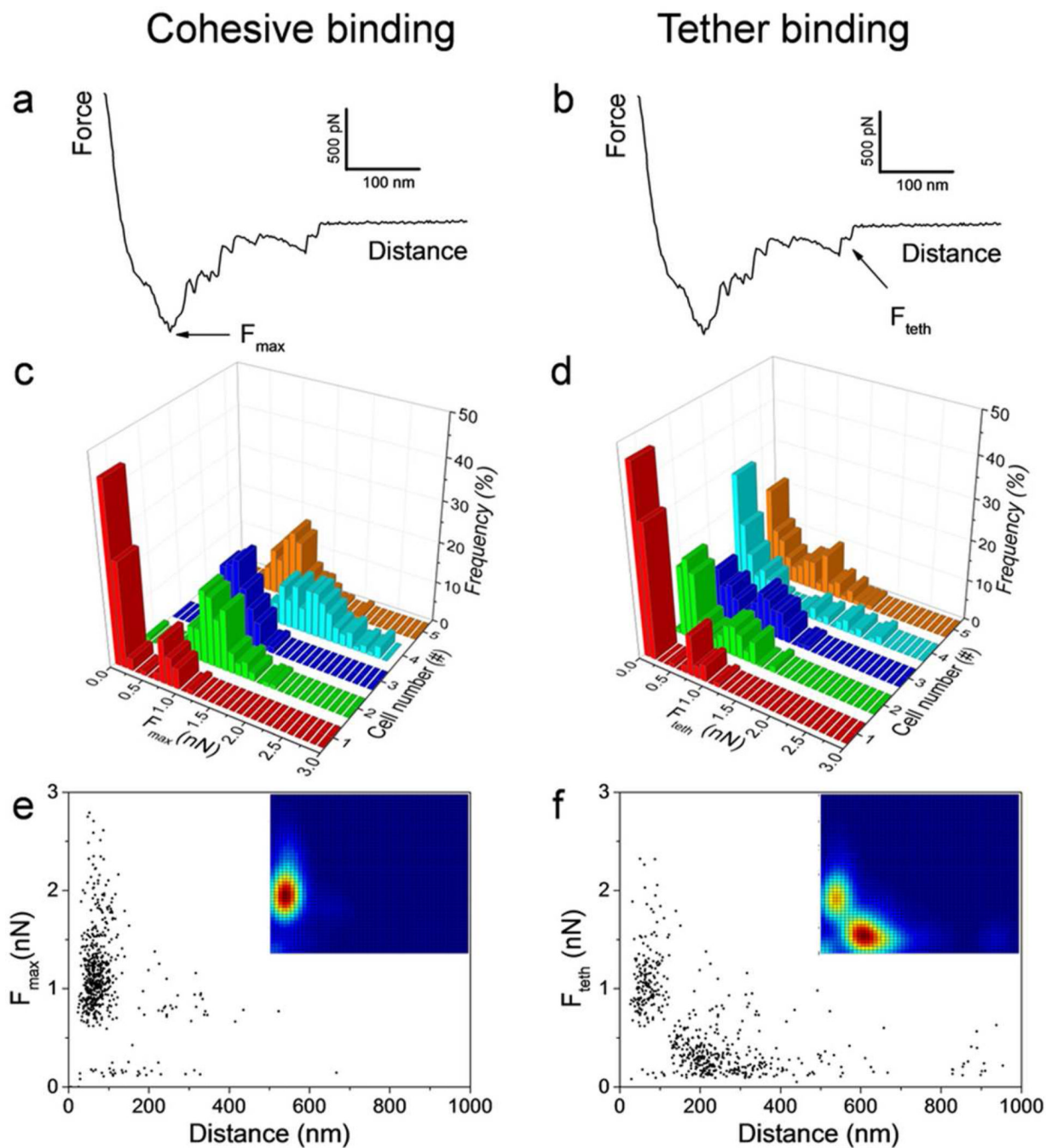
Figure 2.
Force spectroscopy of yeast-hyphae adhesion. (a, b) Representative force curves obtained for the yeast-hyphae interaction showed two characteristic features, i.e. strong, short-range adhesion force peaks (cohesive adhesion, F_{max}), followed by weak, long-range force peaks (tether adhesion, F_{rupt}). (c, d) Adhesion force histograms of the F_{max} and F_{rupt} peaks obtained for 5 different yeast/hyphae combinations ($n = 100$ for each combination). (e, f) Scatter plots of the adhesion force versus distance obtained for F_{max} and F_{rupt} peaks selected randomly from 5 different experiments ($n = 500$ data points). The insets show the

corresponding bivariate color-coded maps of probability function, in which blue and red represent low and high frequency events.
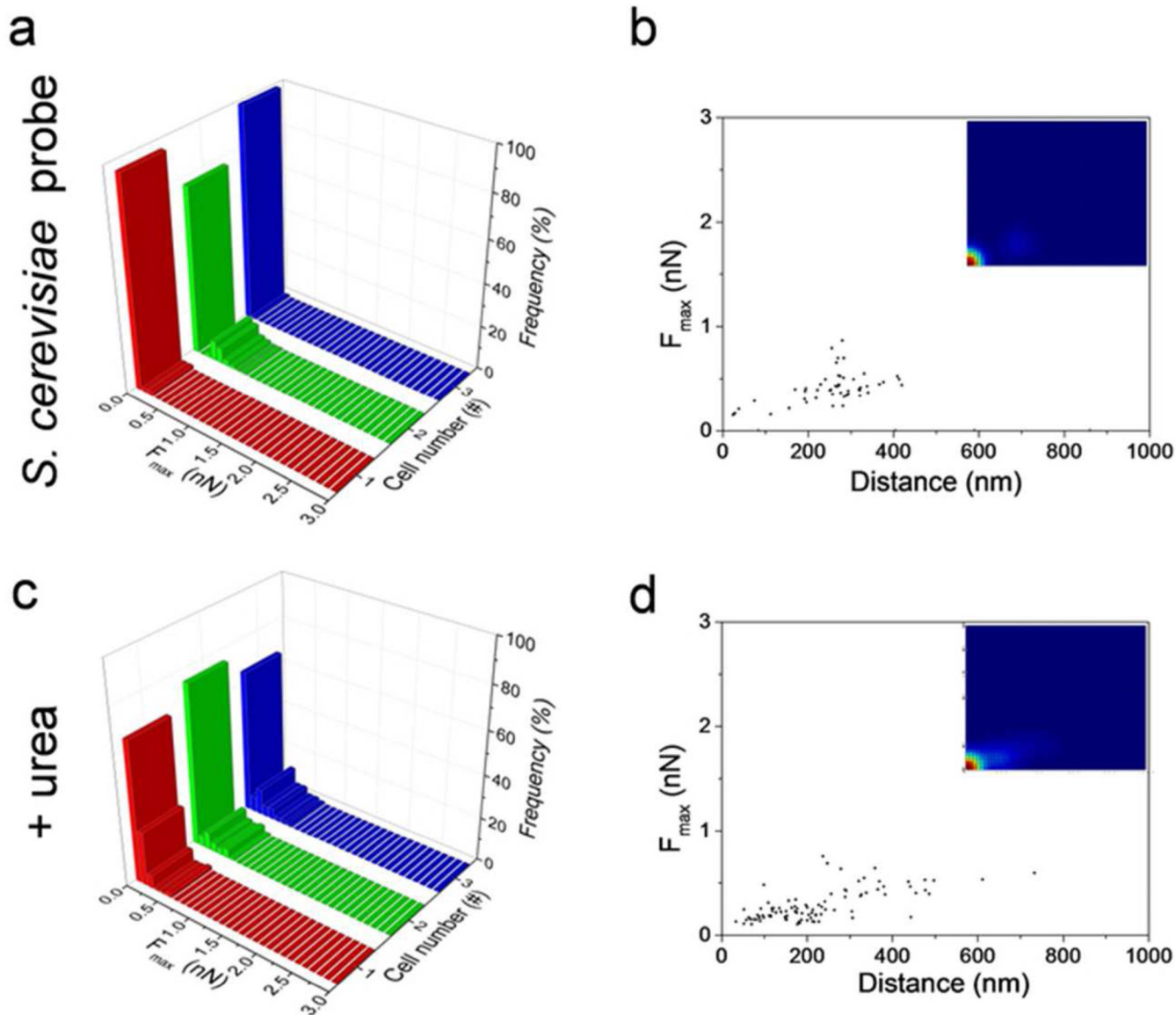
Figure 3.
Yeast-hyphae adhesion involves cell surface proteins that are specific to *C. albicans*. (a, c) Adhesion force histograms and (b, d) scatter plots (*n* = 300 data points; insets: probability density maps) of the strongest adhesion force peaks (F_{max}), obtained for the interaction between *S. cerevisiae* cell probes and *C. albicans* hyphae (a, b) and between native *C. albicans* cell probes and *C. albicans* hyphae treated with 4 M urea (c, d).
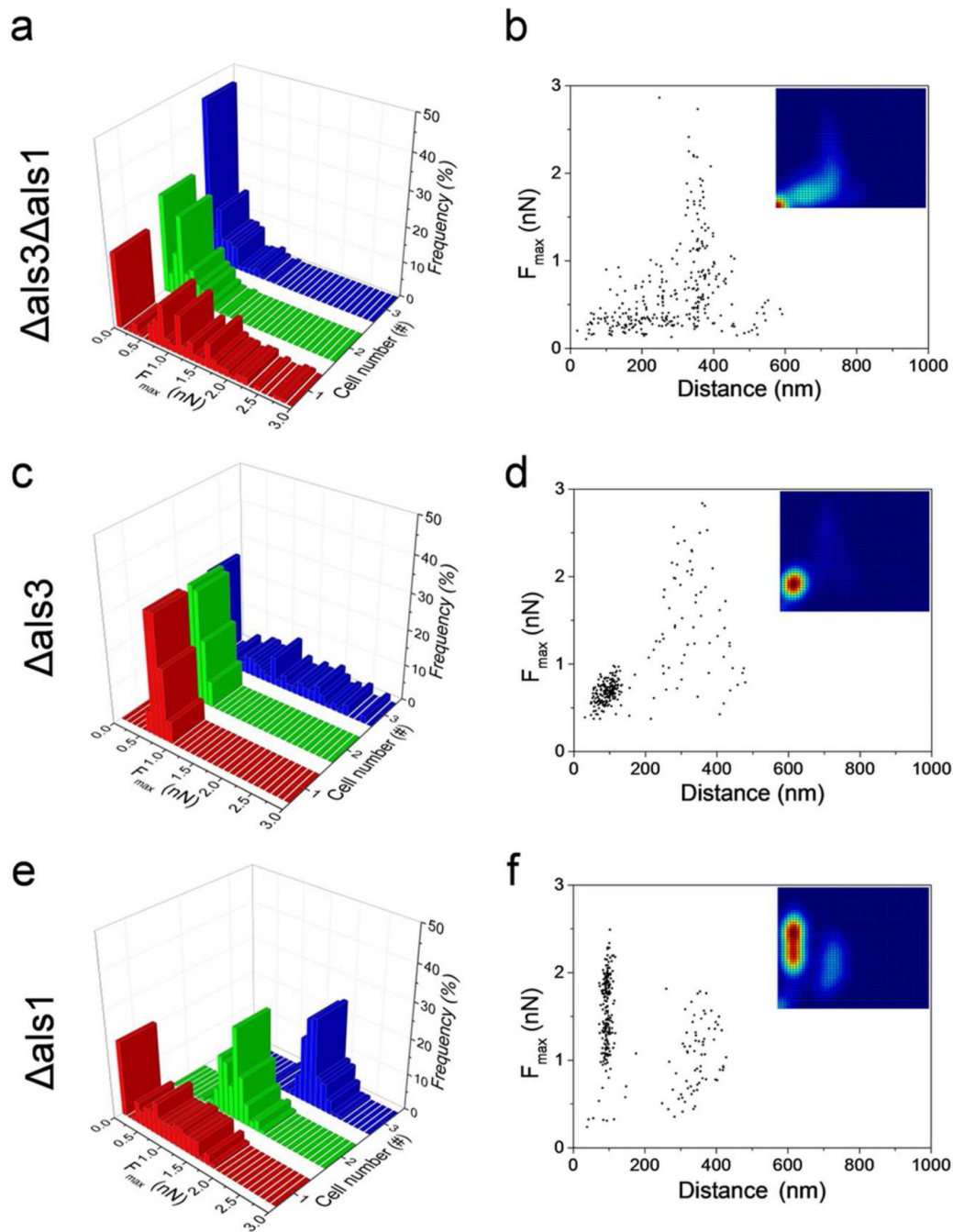
Figure 4.
Role of Als proteins in mediating yeast-hyphae adhesion. (a, c, e) Adhesion force histograms and (b, d, f) scatter plots (n = 300 data points; insets: probability density maps) of the strongest adhesion force peaks (F_{max}) obtained for the interaction between *C. albicans* cell probes and *C. albicans* hyphae from the double mutant *als3Δ/als3Δ als1Δ/als1Δ* (Δals3Δals1) (a, b), and the simple mutants *als3Δ/als3Δ* (Δals3) (c, d) and *als1Δ/als1Δ* (Δals1) (e, f). For each mutant, data from 3 different yeast/hyphae combinations are shown.
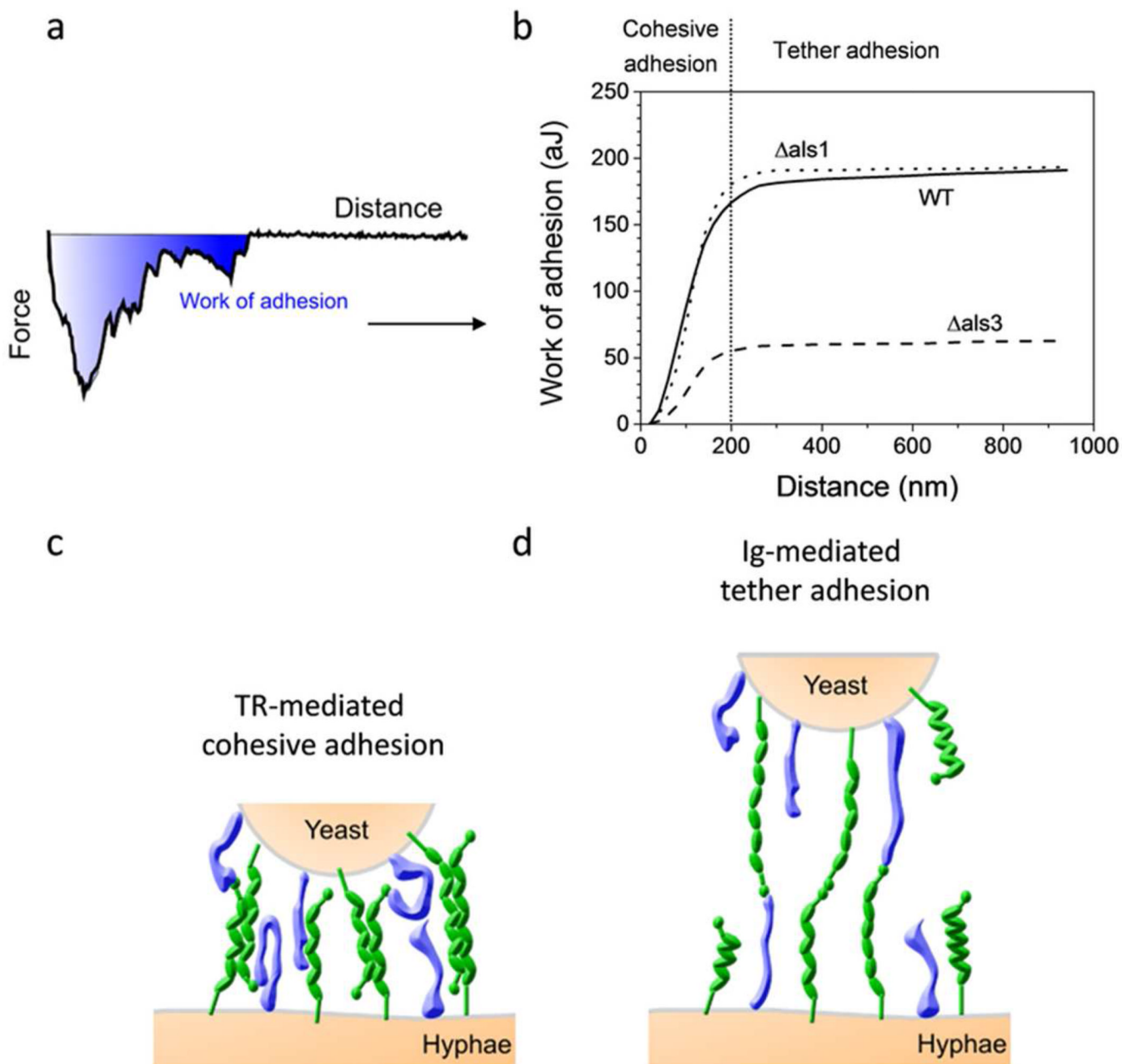
Figure 5.
Mechanism of Als-mediated yeast-hyphal adhesion: interplay of cohesive and tether interactions. (a) To get a molecular view of Als3-mediated cell-cell adhesion, the work of adhesion was calculated from the area under the retraction curves. (b) Variation of the cumulative work of adhesion as a function of the distance, calculated for the WT and for the *als1Δ/als1Δ* (Δals1) and *als3Δ/als3Δ* (Δals3) single mutants. (c, d) Proposed adhesion mechanism in which strong, short-range cohesive interactions are mediated by tight hydrophobic bonds between Als TR domains on adjacent yeast and hyphae (c), while weak, long-range tether interactions involve binding of terminal Ig domains on fully stretched Als to specific ligands (d). Als proteins are shown in green, non-Als proteins such as Hwp1 and Eap1 are shown in blue. The size and surface density of the molecules are not on scale.